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PURINOCEPTORS: ARE THERE FAMILIES OF P2X AND P2Y PURINOCEPTORS?

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Abstract—There has been an exponential growth in interest in purinoceptors since the potent effects of purines were first reported in 1929 and purinoceptors defined in 1978. A distinction between P_1 (adenosine) and P_2 (ATP/ADP) purinoceptors was recognized at that time and later, A_1 and A_2, as well as P_{2X} and P_{2Y} subclasses of P_1 and P_2 purinoceptors were also defined. However, in recent years, many new subclasses have been claimed, particularly for the receptors to nucleotides, including P_{2t}, P_{2z}, $P_{2u(n)}$ and P_{2D}, and there is some confusion now about how to incorporate additional discoveries concerning the responses of different tissues to purines. The studies beginning to appear defining the molecular structure of P_2-purinoceptor subtypes are clearly going to be important in resolving this problem, as well as the introduction of new compounds that can discriminate pharmacologically between subtypes. Thus, in this review, on the basis of this new data and after a detailed analysis of the literature, we propose that:

(1) P2X(ligand-gated) and P2Y(G-protein-coupled) purinoceptor families are established;
(2) four subclasses of P2X-purinoceptor can be identified ($P2X_1$-$P2X_4$) to date;
(3) the variously named P_2-purinoceptors that are G-protein-coupled should be incorporated into numbered subclasses of the P2Y family. Thus:

$P2Y_1$ represents the recently cloned P2Y receptor (clone 803) from chick brain;
$P2Y_2$ represents the recently cloned P_{2u} (or P_{2n}) receptor from neuroblastoma, human epithelial and rat heart cells;
$P2Y_3$ represents the recently cloned P2Y receptor (clone 103) from chick brain that resembles the former P_{2t} receptor;
$P2Y_4$–$P2Y_6$ represent subclasses based on agonist potencies of newly synthesised analogues;
$P2Y_7$ represents the former P_{2D} receptor for dinucleotides.

This new framework for P2 purinoceptors would be fully consistent with what is emerging for the receptors to other major transmitters, such as acetylcholine, γ-aminobutyric acid, glutamate and serotonin, where two main receptor families have been recognised, one mediating fast receptor responses directly linked to an ion channel, the other mediating slower responses through G-proteins. We fully expect discussion on the numbering of the different receptor subtypes within the P2X and P2Y families, but believe that this new way of defining receptors for nucleotides, based on agonist potency order, transduction mechanisms and molecular structure, will give a more ordered and logical approach to accommodating new findings. Moreover, based on the extensive literature analysis that led to this proposal, we suggest that the development of selective antagonists for the different P2-purinoceptor subtypes is now highly desirable, particularly for therapeutic purposes.

Keywords—ATP, P2 purinoceptors, P2X and P2Y, ligand-gated channels, G-protein-linked receptors.

Abbreviations—αβmeATP, α,β-methylene ATP; ADPβS, adenosine 5′-*O*-(2-thiodiphosphate); ANAPP3, 3-*O*-3[*N*-(4-azido-2-nitrophenyl)amino] propionyl ATP; AppNHp, adenosine 5′[β,γ-imido]triphosphate; ApxA, diadenosine polyphosphate; ATPαS, adenosine 5′-*O*-(1-thiotriphosphate); ATPγS, adenosine 5′-*O*-(3-thiotriphosphate); βγmeATP, β,γ-methylene ATP; CF, cystic fibrosis; DAG, diacyl-glycerol; 2meSATP, 2-methylthio-ATP; IP3, inositol-1,4,5-trisphosphate; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid.

CONTENTS

1. EARLY HISTORY OF RECEPTORS TO NUCLEOSIDES AND NUCLEOTIDES

1.1. P_1 and P_2 Purinoceptors

The potent extracellular actions of purine nucleotides and nucleosides, namely adenosine and ATP, were first recognised by Drury and Szent-Gyorgyi (1929) in a seminal paper reporting the activities of adenine compounds on the mammalian heart. Following this report, there was considerable activity within the field, with particular emphasis on the actions of adenosine and ATP on the cardiovascular system (Gillespie, 1933; Green and Stoner, 1950).

The potent vasodilator actions of adenyl compounds led Holton and Holton (1954) to suggest that ATP might represent the vasodilatory substance that was released upon antidromic stimulation of sensory nerves supplying the rabbit ear artery, a hypothesis that was later demonstrated following stimulation of the great auricular nerve (Holton, 1959). The sensitivity of the coronary vasculature to these same compounds prompted Berne (1963) to propose that adenosine was the physiological mediator of the coronary vasodilation associated with myocardial hypoxia, which further supported the role of ATP and adenosine in physiological regulatory processes.

In the same period (early 1960s), a component that was neither adrenergic nor cholinergic was recognised in the autonomic nervous system. This component was strongly represented in the gastrointestinal tract and later identified in a variety of organs, including lung, bladder, seminal vesicles, esophagus, uterus, eye, trachea and part of the cardiovascular system. Using the criteria summarised by Eccles (1964) for the acceptance of putative neurotransmitters, in the early 1970s, Burnstock proposed that the principal active substance released from at least some of these nerves was ATP and, as a consequence, these nonadrenergic, noncholinergic nerves were tentatively termed 'purinergic' (Burnstock *et al.*, 1970; Burnstock, 1972).

In the subsequent years, the purinergic theory was strengthened by a great body of experimental evidence supporting the role of ATP as a transmitter or co-transmitter with noradrenaline,

acetylcholine and other substances (Burnstock, 1976, 1990) and by the identification of specific extracellular receptors mediating the variety of physiological effects induced by purines.

Based largely on an analysis of the voluminous literature about the actions of purines on a wide number of tissues, in 1978, in a seminal review, Burnstock proposed a basis for distinguishing two types of purinergic receptors, terming P_1 and P_2 the purinoceptor preferentially activated by adenosine and ATP, respectively. The original classification into P_1 and P_2 purinoceptors was based on four criteria: (1) the relative potencies of ATP, ADP, AMP and adenosine; (2) the selective actions of antagonists, particularly methylxanthines, which competitively antagonise adenosine, but not ATP actions; (3) the modulation of adenylate cyclase with resultant changes in intracellular cAMP levels by adenosine, but not ATP; and (4) the induction of prostaglandin synthesis by ATP, but not by adenosine. Since the time of this proposal, many experiments have been carried out that support and extend the P_1/P_2 classification (Williams, 1987; Burnstock, 1991), which is now well-established and largely used in the literature and has been adopted by the IUPHAR Subcommittee for Purinoceptor Subclassification (Abbracchio *et al.*, 1993; Fredholm *et al.*, 1994).

In general, the prejunctional purinoceptors that modulate release of noradrenaline from postganglionic sympathetic nerves are P_1. Westfall *et al.* (1990) have proposed the existence of a third purinoceptor subtype on some sympathetic nerve terminals tentatively named P_3 and claimed to recognise the structure of both nucleotides and nucleosides; however, this proposal has not received wide acceptance as yet.

1.2. Subclasses of the Adenosine/P_1 Purinoceptor

Following Burnstock's proposal, subsequent work led to the demonstration of both adenosine/P_1 and ATP/P_2 purinoceptor subtypes.

Parallel work by Londos *et al.* (1980) and Hamprecht's group (van Calker *et al.*, 1979) resulted in the identification of two subclasses of adenosine receptors, which were originally defined on the basis of whether their activation inhibited or stimulated adenylate cyclase activity. Londos *et al.* termed these receptors R_i and R_a based on the need of an intact ribose ring in the purine, with the subscripts 'i' and 'a' referring to inhibition and activation of cAMP formation, respectively, whereas these same two receptors were named A_1 and A_2 by van Calker *et al.*, a nomenclature that found preference in the subsequent literature (Abbracchio *et al.*, 1993).

More recently, A_3 and A_4 receptor subtypes have been identified by cloning (Zhou *et al.*, 1992) and binding studies (Cornfield *et al.*, 1992), respectively. A detailed analysis of experimental evidence supporting the subclassification of the P_1 purinoceptor and of the pathophysiological roles of the different receptor subtypes is not within the aims of the present review, and the reader is referred to excellent reviews recently published in this field (Jacobson *et al.*, 1992; Linden *et al.*, 1994).

1.3. Subclasses of the ATP/P_2 Purinoceptor

Burnstock and Kennedy (1985) provided evidence for a subclassification of the P_2 receptor into the P_{2X} and P_{2Y} subtypes. In this first in-depth review of the functional effects of various ATP analogues in a number of different biological systems, they discriminated between two major classes of receptor-mediated responses on the basis of a different response profile to ATP analogs and selective antagonism. Thus, for P_{2X} purinoceptors, ATP analogs may be listed in order of potency as follows: $\alpha\beta$meATP $\geq \beta\gamma$meATP $>$ ATP $\approx$ 2meSATP $=$ ADP; 3-*O*-3[*N*-(4-azido-2-nitrophenyl)amino] proprionyl ATP (ANAPP3) was claimed to behave as a selective antagonist, whereas prolonged exposure to $\alpha\beta$-methylene ATP ($\alpha\beta$meATP) selectively desensitises this receptor (Kasakov and Burnstock, 1983). For P_{2Y}-purinoceptors: 2meSATP $>$ ATP $\gg$ $\alpha\beta$meATP $=$ $\beta\gamma$meATP; reactive blue 2, an anthraquinone sulfonic acid derivative, has been claimed to be a selective antagonist, at least over a limited concentration range (Kerr and Krantis, 1979; Manzini *et al.*, 1986; Houston *et al.*, 1987).

Studies of the pharmacological actions of isopolar phosphonate analogues of ATP in guinea-pig

taenia coli and bladder have supported the P_{2X}/P_{2Y} subclassification of P_2 purinoceptors in smooth muscle and have also shown that $\beta\gamma$-methylene ATP ($\beta\gamma$meATP) and its analogues behave as selective P_{2X} agonists (Cusack *et al.*, 1987), while adenosine 5′-*O*-(2-thiodiphosphate) (ADPβS) is a specific agonist at P_{2Y}-purinoceptors (Hourani *et al.*, 1988). More recently, the trypanoside suramin has been shown to be a specific antagonist for P_2-purinoceptors, although not selective for the P_{2X}- and P_{2Y}-subclasses (Hoyle *et al.*, 1990), whereas the new compound pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) synthesised by Lambrecht's group appears to represent a selective P_{2X} antagonist (Lambrecht *et al.*, 1992; Ziganshin *et al.*, 1993, 1994b).

After Burnstock and Kennedy's proposal, in 1986, Gordon further delineated ATP effects in a variety of tissues (Gordon, 1986) and defined two additional P_2 purinoceptor subtypes on platelets and mast cells (and lymphocytes) that did not seem to fit the P_{2X}/P_{2Y} subclassification and were tentatively termed P_{2t} and P_{2z} purinoceptors, respectively. The platelet receptor is unique, being activated by ADP and blocked by ATP, whereas the mast cell and macrophage ATP receptor seems to be preferentially activated by the tetrabasic form of ATP, ATP^{4-}, and irreversibly inhibited by oxidised ATP (Murgia *et al.*, 1993). Doubts have been raised about the actual 'receptor' nature of the P_{2z} purinoceptor subtype, since it is represented by the opening of a fairly nonselective membrane pore. Some of the characteristics of the different P_2 purinoceptor subtypes are summarised in Table 1.

From studies performed after Burnstock and Kennedy's proposal, it was evident that the P_{2X} and P_{2Y} purinoceptors could also be differentiated on the basis of their transduction mechanisms. The P_{2X} purinoceptor subtype involves an intrinsic ion channel permeable to Na^+, K^+ and Ca^{2+} (Benham and Tsien, 1987; Bean, 1992), whereas the P_{2Y} purinoceptor subtype is a G-protein coupled receptor that modulates membrane phosphoinositide metabolism and, hence, inositol-1,4,5-trisphosphate (IP3) and diacyl-glycerol (DAG) generation (Cusack, 1993), although additional transduction mechanisms also seem to be utilised in some tissues, such as modulation of cAMP generation (Okajima *et al.*, 1989; Yamada *et al.*, 1992) and arachidonic acid mobilisation (Bruner and Murphy, 1990).

There is now good evidence in favour of the existence of purinoceptors that respond to the pyrimidine derivative UTP, as well as to ATP and adenosine 5′-*O*-(3-thiotriphosphate) (ATPγS) (but not to 2meSATP or $\alpha\beta$meATP, Table 1). As for the P_{2Y} and P_{2t} subtypes, this receptor is linked to G-protein activation (Table 1) and has been alternatively termed 'P_{2u}' or 'nucleotide' or 'pyrimidine' receptor. At this receptor subtype, UTP is generally either equipotent or more potent than ATP, suggesting that the 'pyrimidine'/'nucleotide' receptor might indeed represent a further subclass of the P_2 purinoceptor (see also Section 2).

Besides these P_2 purinoceptor subtypes, there also appear to be receptors for adenine dinucleotide polyphosphates (Ap4A, Ap5A and Ap6A), leading to the proposal of an additional receptor subtype, the 'P_{2D}' purinoceptor (Hilderman *et al.*, 1991; Castro *et al.*, 1992; Table 1). The physiological functions of the diadenosine polyphosphate (ApxA) compounds are unclear at present, although possible roles as neurotransmitters, co-transmitters or trophic factors have been suggested (Hoyle, 1990).

The use of different terminologies to define UTP-sensitive responses and the apparent random walk through the alphabet in the naming of P_{2n}, P_{2t}, P_{2u} and P_{2z} and P_{2D} has generated some concern in the IUPHAR Subcommittee for Purinoceptor Subclassification (Abbracchio *et al.*, 1993; Fredholm *et al.*, 1994), and has led to confusion and inconsistency, especially when trying to incorporate into current nomenclature schemes additional discoveries about the biological responses to purines and pyrimidines.

This confusion has prompted us to perform a detailed analysis of the available literature in order to verify whether a new way of defining the different receptor subtypes for nucleotides could be proposed. Knowledge of receptor molecular structure is critical and strategic in defining neurotransmitter receptor subtypes. Since molecular biology data on P_2 purinoceptors are now becoming available, we would like to summarise initially such available information in order to try to identify some useful criteria in P_2 purinoceptor subclassification. Further, we report here the results of studies recently performed with a number of modified ATP analogues that give further hints about possible P2X- and P2Y-purinoceptor subclasses.

Table 1. *P_2 Purinoceptor: Characteristics and Current Subclassification*

Receptor	P_{2X}	P_{2Y}	P_{2u} (P_{2n})	P_{2t}	P_{2z}	P_{2D}
Type	Intrinsic ion channel (Na^+, K^+, Ca^{2+})	G-protein-coupled (IP3/Ca^{2+}/DAG)	G-protein-coupled (IP3/Ca^{2+}/DAG)	G-protein-coupled (IP3/Ca^{2+}/ DAG/ cAMP)	Nonselective pore	G-protein-coupled (IP3/Ca^{2+}/D AG)
Agonist profile	$\alpha\beta$meATP $\geq$ $\beta\gamma$me ATP > ATP $\geq$ 2meSATP $\approx$ ADP	2meSATP $\gg$ ATP $\gg$ $\alpha\beta$meATP	UTP $\geq$ ATP $\gg$ 2meSATP	2-ADP	ATP^{4-}	ApxA
Antagonist	Desensitisation by $\alpha\beta$meATP; blocked by suramin, by ANAPP3 and PPADS	Blocked by suramin and by reactive blue 2		ATP	Oxidised ATP	

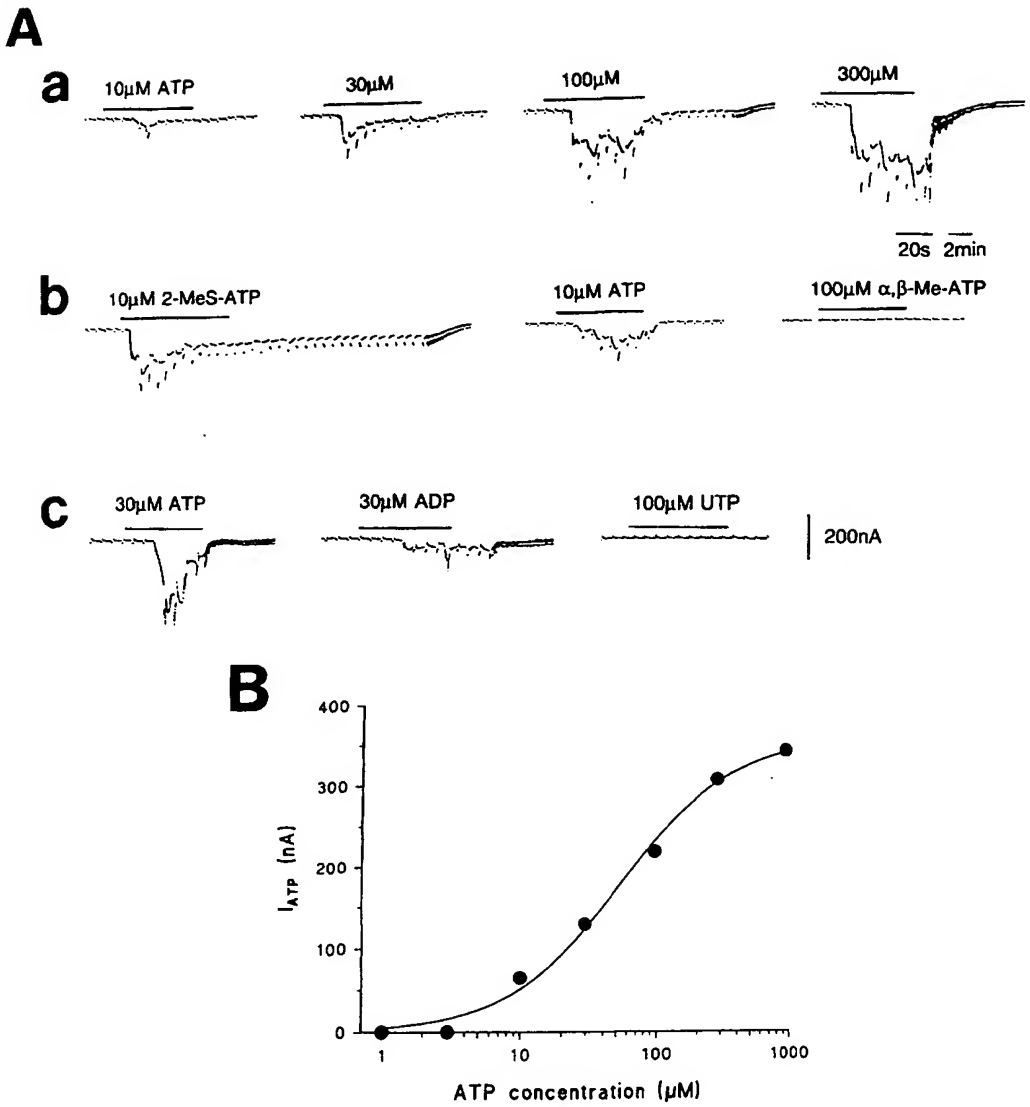

Fig. 1(A, B)—see opposite.

2. MOLECULAR BIOLOGY OF ATP PURINOCEPTORS

Acquired ATP-generated currents in *Xenopus* oocytes injected with mRNA from embryonic guinea-pig brain were first demonstrated by Fournier *et al.* (1990), and by Honoré *et al.* (1991), and also following injection of size-fractionated RNA from J774 murine macrophage-like cells by Hickman *et al.* (1993). ATPγS-induced acceleration of intracellular calcium efflux was shown in *Xenopus* oocyte injected with mRNA from HL60 promyelocytic leukaemia cells, a response that is believed to be mediated by a G-protein-linked P_2 purinoceptor (Murphy and Tiffany, 1990). More recently, Russell *et al.* (1993) reported ATP-generated currents in *Xenopus* oocytes injected with poly-$(A)^+$ mRNA from guinea-pig vas deferens. Such ATP-generated currents involved the release of intracellular Ca^{2+} and also depended upon Ca^{2+} influx.

The Barnard and Burnstock groups have recently cloned a P_{2Y}-like purinoceptor using a homology screening strategy. A previously isolated guinea-pig partial cDNA,* the sequence homologue

*Webb, T. E., Bateson, A. N. and Barnard, E. A. (1991) A polymerase chain reaction based strategy for the isolation of DNA sequences that encode G-protein-coupled receptors. In: *17th EMBO Annual Symposium, Molecular Mechanisms of Signal Transduction*, Heidelberg, Germany, 16–19 September, 1991, Abstract No. 208.

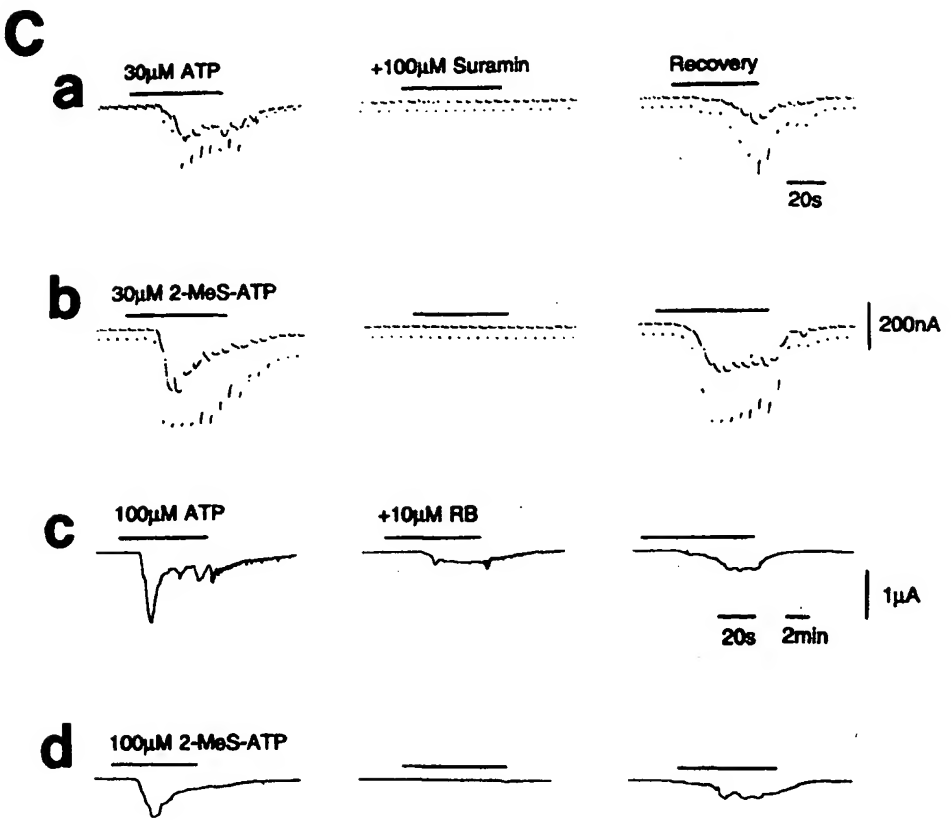

Fig. 1(C).

Fig. 1. Responses in cRNA-injected *Xenopus* oocytes (at −40 mV). **Aa**: Dose-dependence of membrane currents evoked by ATP (10–300 μM). The transient downward deflections monitor the input conductance following hyperpolarising voltage steps (−10 mV, applied every 5 sec for 1 sec). Similar results were observed in two other oocytes. **Ab,Ac**: In two oocytes, the agonist selectivity was assessed using 2meSATP, ATP, ADP, αβmeATP and UTP. Holding potentials, −40mV. **B**: ATP concentration–response relationship. The membrane current amplitude (I_{ATP}) was measured using the initial peak inward current induced by each ATP concentration. **C**: Suramin antagonised the responses to (**Ca**) ATP and (**Cb**) 2meSATP. Reactive blue 2 (RB) also inhibited the responses to (**Cc**) ATP and (**Cd**) 2meSATP. Bar indicates ligand application. Adapted from Webb *et al.* (1993).

of the canine orphan receptor RDC1 (Libert *et al.*, 1989), was used to screen an embryonic chick whole brain cDNA library under conditions of low stringency (Webb *et al.*, 1993). The cloning strategy included use of two degenerate oligonucleotide primers from transmembrane domains II and VI of G-protein-coupled receptors for polymerase chain reaction amplification of guinea-pig brain first-strand cDNA, subcloning of amplification products and screening of an embryonic chick whole brain cDNA library.

An isolated clone, 803, encoded a 362-amino acid polypeptide displaying the typical topology of a G-protein-coupled receptor (Webb *et al.*, 1993), such as the presence of seven hydrophobic putative transmembrane α-helices; consensus sequences for N-linked glycosylation in the N-terminus extracellular domain; two conserved cysteine residues in the first and second extracellular loops, believed to form disulphide bonds; and several consensus phosphorylation sites in the third cytoplasmic loop and C-terminal intracellular domain (Linden *et al.*, 1991).

Functional expression of this clone in *Xenopus laevis* indicated that the encoded protein is a P_2-purinoceptor. ATP induced a large calcium-activated inward chloride current (Webb *et al.*, 1993; Fig. 1Aa). Responses to ATP were dose-dependent with an EC_{50} of 49.5 ± 6 μM (Fig. 1Aa,B). Further analysis of the ligand specificity of this receptor using a range of P_2-purinoceptor agonists led to designation of this clone as a P2Y-like purinoceptor. ATP-like effects were also induced by 2meSATP

and ADP, whereas both UTP and the P_{2X} selective agonists $\alpha\beta$meATP and $\beta\gamma$meATP were inactive at concentrations up to 30–100 μM. Therefore, the rank order of potency of these agonists was: 2meSATP $\geq$ ATP > ADP $\gg$ $\alpha\beta$meATP, $\beta\gamma$meATP, UTP (Fig. 1Ab,Ac). Both suramin and the P_{2Y}-selective antagonist reactive blue 2 antagonised the responses to ATP and 2meSATP (Fig. 1C). Based on selective antagonism by reactive blue 2, high potency of ATP, inactivity of UTP and methylene ATP derivatives, and strong structure similarity with G-protein-coupled receptors, the newly cloned receptor was identified as a P_{2Y} subtype rather than a P_{2X} or P_{2u}/'nucleotide' purinoceptor subtype. Moreover, the near equipotency of 2meSATP and ATP and greater potency of ATP over ADP suggested that this expressed receptor was a novel subtype of the P2Y purinoceptor family, which was consequently named '$P2Y_1$'.

The agonist profile typical of this receptor (2meSATP $\geqslant$ ATP > ADP) was a starting point for us in analyzing the available literature in our effort to find a new and logical way of defining the different receptor subtypes to ATP (see Section 4.4).

The cloning work by the Barnard and Burnstock groups also led to the isolation of an ADP-sensitive clone (clone 103), which, based on preliminary pharmacological results, might be related to the so-called 'P_{2t}' purinoceptor (Barnard *et al.*, 1994).

Lustig *et al.* (1993) have independently cloned a cDNA from NG 108-15 neuroblastoma cells encoding a metabotropic P_{2u}/'nucleotide' purinoceptor by utilising a *X. laevis* oocyte expression cloning strategy. In *Xenopus* oocytes injected with poly(A)$^+$ RNA from NG 108-15 cells, ATP or UTP (1 mM) evoked calcium-dependent inward currents. To identify the cDNA encoding the receptor protein responsible for these currents, pools of 2×10^5 individual clones from an NG 108-15 cDNA plasmid library were *in vitro* transcribed and the resultant cRNA was injected into oocytes. The pool that rendered the oocytes responsive to ATP or UTP was subdivided progressively and the procedure was repeated until a single clone, pP2R, was obtained (Lustig *et al.*, 1993).

In oocytes injected with pP2R cRNA transcripts, inward currents were elicited by bath application of ATP, UTP or ATPγS, but not by comparable concentrations of 2meSATP, ADP, $\beta\gamma$meATP or $\alpha\beta$meATP. The order of agonist potency was ATP = UTP > ATPγS $\gg$ 2meSATP, ADP, $\beta\gamma$meATP, $\alpha\beta$meATP, which, again, was a starting point for us in analysing the effects reported in the literature for ATP and UTP.

Recently, the ATP/UTP receptor has been cloned from human airway and colonic epithelium (Parr *et al.*, 1994) and from rat heart (Godecke and Schrader, 1994). Similarly to the receptor cloned by Lustig *et al.*, the HP2U clone isolated by Parr *et al.*, when expressed in 1321N1 astrocytoma cells, displayed comparable responses to ATP and UTP, whereas 2meSATP and $\alpha\beta$meATP had little effect. Activation of this receptor leads to breakdown of phosphoinositides and increase of intracellular Ca^{2+}, an effect that was partially blocked by pertussis toxin.

Both the $P2Y_1$-purinoceptor and the cloned pP2R and HP2U-purinoceptors showed predicted structural features characteristic of most known G-protein-coupled receptors.

Figure 2 shows the deduced protein sequences of cloned $P2Y_1$ and pP2R purinoceptors in comparison with adenosine A_1, A_{2a}, A_{2b} and A_3 receptors. Interestingly, the $P2Y_1$ and pP2R sequences differ markedly from the cloned adenosine receptors. In fact, the pP2R receptor is more similar to receptors for various peptides, such as thrombin (25% identity), platelet-activating factor (25% identity), angiotensin II (22% identity), interleukin 8 (23% identity) and GPRN1, a putative vasoactive intestinal peptide receptor (21% identity) (Lustig *et al.*, 1993). The cloned receptor is substantially less similar (< 12% identity) to adenosine and cAMP receptors.

Similarly, the $P2Y_1$ receptor has only a low sequence identity with the adenosine A_1 (21%) and the cAMP (17%) receptors, whereas, as reported for the pP2R receptor, higher identities were found with the angiotensin II, thrombin, platelet-activating factor and interleukin 8 receptors (Table 2), suggesting that these two purinoceptors might belong to a completely different subfamily of the G-protein-coupled receptor class.

The tissue distribution of the $P2Y_1$ transcript determined by Northern hybridisation revealed a discrete pattern of expression in the adult chicken. The $P2Y_1$ mRNA was present in brain, spinal cord, gastrointestinal tract, spleen and leg muscle (Webb *et al.*, 1993). Conversely, for the pP2R receptor, Northern blot analysis revealed wide distribution of mRNA in all tested mouse tissues, such as spleen, testes, kidney, liver, lung, heart and brain (Lustig *et al.*, 1993). This distribution is very similar to that reported for the HP2U receptor (Parr *et al.*, 1994). The distribution of the $P2Y_1$ (Webb


A
TM 1
TM 2
A1-RECEPTORS
hA1 MPPSISAFQAAYIGIEVLIALVSVPGNVLVIWAVKVNQALRDATFCFIVSLAVADVAVGALVIPLAILINIGPQTYFHTC
rA1 MPPYISAFQAAYIGIEVLIALVSVPGNVLVIWAVKVNQALRDATFCFIVSLAVADVAVGALVIPLAILINIGPQTYFHTC
dA1 MPPAISAFQAAYIGIEVLIALVSVPGNVLVIWAVKVNQALRDATFCFIVSLAVADVAVGALVIPLAILINIGPRTYFHTC
bA1 MPPSISAFQAAYIGIEVLIALVSVPGNVLVIWAVKVNQALRDATFCFIVSLAVADVAVGALVIPLAILINIGPRTYFHTC
A2-RECEPTORS
hA2a MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNVTNYFVVSLAAADIAVGVLAIPFAITISTGFCAACHGC
dA2a MSTMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNVTNYFVVSLAAADIAVGVLAIPFAITISTGFCAACHNC
rA2a MGSSVYITVELAIAVLAILGNVLVCWAVWINSNLQNVTNFFVVSLAAADIAVGVLAIPFAITISTGFCAACHGC
hA2b MLLETQDALYVALELVIAALSVAGNVLVCAAVGTANTLQTPTNYFLVSLAAADVAVGLFAIPFAITISLGFCTDFYGC
rA2b MQLETQDALYVALELVIAALAVAGNVLVCAAVGASSALQTPTNYFLVSLATADVAVGLFAIPFAITISLGFCTDFHSC
A3-RECEPTORS
rA3 MKANNTTTSALWLQITYVTMEAAIGLCAVVGNMLVIMVVKLNRTLRTTTFYFIVSLALADIAVGVLVIPLAIAVSLEVQMHFYAC
P2 - RECEPTORS
P2Y1 MTEALISAALNGTQPELLAGGMAAGNATTECSLTKTGFQFYYLPTVYILVFITGFLGNSVAIWMFVFHMRPWSQISVYMFNLALADFLYVLTLPALIFYYFNKTDWIFGDVMCK
P2U MAADLEPWNSTINGTWEGDELGYKCRFNEDFKYVLLPVSYGVVCVLGLCLNVVALYIFLCRLKT-WNASTTYMFHLAVSDSLYAASLPLLVYYYARGDHWPFSTVLCK
TM 3
TM 4
TM 5
hA1 LMVACPVLILTQSSILALLAIAVDRYLRVKIPLRYKMVVTPRRAAVAIAGCWILSFVVGLTPMFGWN.....NLSAVERAWAANGSMGEPVIKCEFEKVISMEYMVYFNFFVWVLPP
rA1 LMVACPVLILTQSSILALLAIAVDRYLRVKIPLRYKTVVTQRRAAVAIAGCWILSLVVGLTPMFGWN.....NLSVVEQDWRANGCVGEPVIKCEFEKVISMEYMVYFNFFVWVLPP
dA1 LMVACPVLILTQSSILALLAIAVDRYLRVKIPLRYKTVVTPRRAAVAIAGCWILSFVVGLTPLFGWN.....RLGEAQRAWAANGSGGEPVIKCEFEKVISMEYMVYFNFFVWVLPP
bA1 LKVACPVLILTQSSILALLAIAVDRYLRVKIPLRYKTVVTPPRAVVAITGCWILSFVVGLTPMFGWN.....NLSAVERDWLANGSVGEPVIECQFEKVISMEYMVYFNFFVWVLPP
hA2a LFIACFVLVLTQSSIFSLLAIAIDRYIAIRIPLRYNGLVTGTRAKGIIAICWVLSFAIGLTPMLGWN.....NCGQPKEGKNHSQGCGEGQVACLFEDVVPMNYMVYFNFFACVLVP
dA2a LFFACFVLVLTQSSIFSLLAIAIDRYIAIRIPLRYNGLVTGTRAKGIIAVCWVLSFAIGLTPMLGWN.....NCSQPKEGRNYSQGCGEGQVACLFEDVVPMNYMVYYNFFAFVLVP
rA2a IFFACFVLVLTQSSIFSLLAIAIDRYIAIRIPLRYNGLVTGVRAKGIIAICWVLSFAIGLTPMLGWN.....NCSQKDGNSTKT..CGEGRVTCLFEDVVPMNYMVYYNFFAFVLLP
hA2b LFLACFVLVLTQSSIFSLLAVAVDRYLAICVPLRYKSLVTGTRARGVIAVLWVLAFGIGLTPFLGWNSK..DSATNNCTEPWDGTTNE.SCC..LVKCLFENVVPMSYMVYFNFFGCVLPP
rA2b LFLACFVLVLTQSSIFSLLAVAVDRYLAIRVPLRYKGLVTGTRARGIIAVLWVLAFGIGLTPFLGWNSK..DRATSNCTEPGDGITNK.SCC..PVKCLFENVVPMSYMVYFNFFGCVLPP
rA3 LFMSCVLLVFTEASIMSLLAIAVDRYLRVKLTVRYRTVTTQRRIWLFLGLCWLVSFLVGLTPMFGWNRKVTLELSQNSS.TLS.....CHFRFVVGLDYMVFFSFITWILIP
P2Y1 .LQRFIFHVNLYGSILFLTCISVHRYTGV...VHPLKSLGRLKKKNAVYVSSLVWALVVAVIAPIFYSGTGVRR..NKT.....ITCYDTTADEY....LRSTFVYSMCTTVFMFCIPP
P2U LVRFLFYTNLYCSILFLTCISVHRCLGVL.RPLHSLRWGRA..RYARRVAAVVWVLVLACQAPVLYFVTTSVRGTRITCHDTSARELFS HFVAYSSVMLGLL

Fig. 2(A).

B

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            TM 5                                                  TM 6                                        TM 7
hA1    LLIMVLIYLEVFYLIRKQLNKKVSA..SSGDPQKYYGKELKIAKSLALILFLFALSWLPLHILNCITLFCPSC..HKPSILTYIAIFLTHGNSAMNPIVIAF
rA1    LLIMVLIYLEVFYLIRKQLNKKVSA..SSGDPQKYYGKELKIAKSLALILFLFALSWLPLHILNCITLFCPTC..QKPSILIYIAIFLTHGNSAMNPIVYAF
dA1    LLIMVLIYLEVFYLIRRQLGKKVSA..SSGDPQKYYGKELKIAKSLALILFLFALSWLPLHILNCITLFCPSC..RKPSILMYIAIFLTHGNSAMNPIVYAF
bA1    LLIMVLIYMEVFYLIRKQLSKKVSA..SSGDPQKYYGKELKIAKSLALILFLFALSWLPLHILNCITLFCPSC..HMPRILIYIAIFLSHGNSAMNPIVYAF

hA2a   LLIMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVGLFALCWLPLHIINCFTFFCPDSC.HAPLWLMYLAIVLSHTNSVVNPFIYAY
dA2a   LLIMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVGLFALCWLPLHIINCFTFFCPECS.HAPLWLMYLTIVLSHTNSVVNPFIYAY
rA2a   LLIMLAIYIRIFLAARRQLKQMESQPLPGERTRSTLQKEVHAAKSLAIIVGLFALCWLPLHIINCFTFFCSTCR.HAPPWLMYLAIILSHSNSVVNPFIYAY

hA2b   LLIMLVIYIKIFLVACRQLQRTELM....DHSRTTLQREIHAAKSLAMIVGIFALCWLPVHAVNCVTLFQPAQGKNKPKWAMNMAILLSHANSVVNPIVYAY
rA2b   LLIMMVIYIKIFMVACKQLQHMELM....EHSRTTLQREIHAAKSLAMIVGIFALCWLPVHAINCVTLFHPALAKDKPKWVMNVAILLSHANSVVNPIVYAY

rA3    LVVMCIIYLDIFYIIRNKLSQNLTG...FRETRAFYGREFKTAKSLFLVLFLFALCWLPLSIINFVSYFNV....KIPEIAMCLGILLSHANSMMNPIVYAC

P2Y1   IVILGCYGLIVKALIYKDLDNSP.............LRRKSIYLVIIVLTVFAVSYLPFHVMKTLNLRARL..DFQTPQMCAFNDKVYATYQVTRGLASLNSCVDPILYFLAG
P2U    FAVPFSVILVCYVLMARRLLKPAYGTTGGLPRAKRK..........SVRTIALVLAVFALCFLPFHVTRTLYYSFRSLDLSCHTLNAINMAYKITRPLASANSLDPVLYFLAG

hA1    RIQKFRVTFLKIWNDHFRCQPAPPIDEDLPEERPDD
rA1    RIHKFRVTFLKIWNDHFRCQPKPPIDEDLPEEKAED
dA1    RIQKFRVTFLKIWNDHFRCQPTPPVDEDPPEEAPHD
bA1    RIQKFRVTFLKIWNDHFRCQPAPPIDEDAPAERPDD

hA2a   RIREFRQTFRKIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNGYALGLVSGGSAQESQGNTGLPDVELLSHELKGVCPEPPGLDDPLAQDGAGVS
dA2a   RIREFRQTFRKIIRSHVLRRREPFKAGGTSARALAAHGSDGEQISLRLNGHPPGVWANGSAPHPERRPNGYTLGLVSGGIAPESHGDMGLPDVELLSHELKGACPESPGLEGPLAQDGAGVS
rA2a   RIREFRQTFRKIIRTHVLRRQEPFQAGGSSAWALAAHSTEGEQVSLRLNGHPLGVWANGSATHSGRRPNGYTLGLGGGGSAQGSPRDVELPTQERQEGQEHPGLRGHLVQARVGASS
       WSSEFAPS

hA2b   RIRDFRYTFHKIISRYLLCQADVKSGNGQAGVQPALGVGL
rA2b   RIRDFRYSFHRIISTYVLCQTDTKGGSGQAGGQSTFSLSL

rA3    KIKKFKETYFVILRACRLCQTSDSLDSNLEQTTE

P2Y1   DTFRRRLSRATRKSSRRSEPNVQSKSEEMTLNILTEYKQNGDTSL
P2U    QPLVRFARDAKP
```

Fig. 2. Deduced amino acid sequences of cloned purinoceptors. The transmembrane helices (TM-1 to TM-7) are designated on the basis of hydropathy plots. The human A_1 receptor (Salvatore *et al.*, 1992), the rat A_1 receptor (Mahan *et al.*, 1991), the canine A1 receptor (Libert *et al.*, 1991) and the bovine A_1 receptor (Maenhaut *et al.*, 1990) all have 326 amino acids. The human (Salvatore *et al.*, 1992) and canine (Maenhaut *et al.*, 1990) A_{2a} receptors have 412 and the rat A_{2a} receptor (Fink *et al.*, 1992) 410 amino acids. The rat (Stehle *et al.*, 1992) and human (Salvatore *et al.*, 1992) A_{2b} receptors have 332 amino acids, wheras the rat A_3 receptor (Meyerhof *et al.*, 1991; Zhou *et al.*, 1992) has 319. The P2Y$_1$ receptor has 362 amino acids (Webb *et al.*, 1993) and the putative P_{2U} receptor has 373 amino acids (Lustig *et al.*, 1993). Modified from Fredholm *et al.* (1994).

et al., 1993) and the 'P_{2u}' (Lustig *et al.*, 1993; Parr *et al.*, 1994) purinoceptors is taken into considerations in terms of P2Y purinoceptor subclassification (Section 4.4).

3. NEW ATP DERIVATIVES AS POSSIBLE TOOLS TO DISCRIMINATE P2 PURINOCEPTOR SUBTYPES

The confusion surrounding the classification of ATP receptors is partly due to a multiplicity of biological effects and inconsistencies in the potency of available agonists and antagonists (Silinsky, 1989; Inoue and Nakazawa, 1992). In this respect, many efforts recently have been aimed at synthesizing novel ATP analogues endowed with high selectivity and potency for the different P2 purinoceptor subtypes.

In collaboration with Burnstock's and Harden's laboratories, Jacobson and coworkers recently have designed ATP derivatives with modified purine, ribose or triphosphate moieties and characterised their activity in a variety of pharmacological assay systems known to possess P2X and P2Y-purinoceptors (Fischer *et al.*, 1993; Burnstock *et al.*, 1994). Pharmacological assays at P2Y purinoceptors included: stimulation of the production of IP3 formation in turkey erythrocytes, relaxation of guinea-pig taenia coli and of rabbit mesenteric artery smooth muscle, and endothelium-dependent relaxation of rabbit aorta. Pharmacological assays at P2X purinoceptors included contraction of the rabbit saphenous artery and contraction of the guinea-pig vas deferens and urinary bladder.

This data is summarised in Table 3, which reports the EC_{50} or pD2 values ($-\log EC_{50}$) of all the compounds tested in the pharmacological assays. Some analogs displayed selectivity or specificity for either the P2X or P2Y purinoceptor in selective tissues, suggesting heterogeneity within these two purinoceptor families.

Consistent with data showing enhanced potency at P2-purinoceptors of long chain functionalised congeners of 2meSATP (Zimmet *et al.*, 1993), 2-alkylthio derivatives of ATP proved to be the most potent compounds in all the P2Y-purinoceptor assays (Table 3). Indeed, both base modifications, leading to 8-(6-aminohexylamino)ATP and *N*-oxide ATP, and ribose modifications, leading to 2′,3′-isopropylidene-AMP, resulted in potent derivatives that displayed selectivity for endothelial P2Y purinoceptors and were practically inactive in the other P2Y- and P2X-purinoceptor pharmacological assays (Table 3). Conversely, the potent agonist N^6-methyl-ATP and the somewhat less potent agonist 2′-deoxy-ATP were selective for guinea-pig taenia coli P2Y purinoceptors, but were inactive at the vascular P2Y and at the P2X purinoceptors. These results suggest

Table 2. *Percentage Identity of Chick $P2Y_1$ Purinoceptor with known G-Protein-Coupled Receptors*

Receptor	Species	%Identity with $P2Y_1$
RDC1	Canine	27
Angiotensin II type 1	Human	27
Thrombin	Human	25
Platelet activating factor	Guinea-pig	25
C5a anaphylatoxin	Human	23
Neuromedin K	Rat	23
Interleukin 8	Human	22
Bradykinin B2	Rat	22
Neurotensin	Rat	21
Endothelin B	Human	21
Gastrin-releasing peptide	Mouse	21
Adenosine A_1	Canine	21
Substance P	Human	20
Neurokinin 2	Human	20
Adenosine A_2	Canine	18
cAMP	Slime mold	17

The most related sequences are shown, along with adenosine and cAMP receptor. RDC1, canine orphan receptor RDC1 (Libert *et al.*, 1989). Adapted from Webb *et al.* (1993).

Table 3. *Activity of Nucleotide Analogs in Various Biochemical and Pharmacological Models*

	P2Y purinoceptors				P2X purinoceptors		
	Biochemical assay	Mediating relaxation (relative to ATP)[2]			Mediating contraction (relative to ATP)[2]		
Compound	Turkey erythrocyte[1]	Guinea-pig taenia	Rabbit aorta	Rabbit mesenteric artery	Rabbit saphenous artery[3]	Guinea-pig vas deferens	Guinea-pig bladder
ATP and triphosphate modifications							
1. ATP	2800 ±700	= (6.2)[4]	= (4.5)	= (6.0)	= [5%]	= (3.5)[5]	=[5]
2. ADP	8000 ±2000	=[5]	= (5.2)	= (5.2)	na	− −[5]	=[5]
3. AMP	4 ±2% at 10^{-4} M	− −[5]	= (4.8)	= (5.0)	na	− −	na[5]
4. αβmeATP	≫100,000	= (5.6)[5]	See footnote 6	See footnote 6	= [5.9%]	+ (5.3)[5]	+ + (5.7)[5]
5. βγmeATP	≫100,000	− −[5]	See footnote 6	See footnote 7	+ + [89%]	+[5]	+ +[5]
6. βγme-3′,5′-cyclic ATP	na	− −	na	na	na	na	na
7. AppNHp	4,450 ±1,150	− −[5]	+ (5.5)	+ (6.6)	na	+	+
8. ATPαS (S-isomer)	8,930 ±4,440	+[5]	See footnote 6	+ (6.6)	na	=[5]	=[5]
9. ATPγS	1,260 ±380	=[5]	+ (5.7)	+ (5.8)	na	+ +[5]	+
10. ADPβS	96 ±27	=	+ (5.8)	+ (5.8)	na	=[5]	=[5]
Base modifications							
11a. 2meSATP	8 ±2	+ + (8.0)	+ + (6.8)[5]	+ + (6.5)	na	=[5]	=[5]
11b. 2-(6-cyano-hexylthio)-ATP	10 ±5	+ + (8.8)	+ + (6.9)	+ + (7.0)	= [9.2%]	+ +	See footnote 8
12. N^6methyl-ATP[5]	19,000 ±6,000	+ (5.8)	− −	na	na	na	na[5]
13. 8-Bromo-ATP	47,000	− −	na	na	na	na	−
14. 8-(6-Amino-hexylamino)-ATP	8,200 ±1,200	− −	+ + (7.3, <max)	na	na	na	na
15. Adenosine N1-oxide 5′-triphosphate	16,900 ±4,900	− (4.9)	+ + (6.7, >max)	na	na	na	na
16. N1,N^6-etheno-ATP	≫100,000	− −	na	na	na	na	−
17. UTP	143,000 ±44,000	− − (3.5)	= (4.8)	+ (6.7)	na	na	=[5]
18. 5-Fluoro-UTP	≫100,000	− −	+ (6.0, ≈max)	na	na	na	+ +

Table 3—*Continued*

Compound	P2Y purinoceptors				P2X purinoceptors		
	Biochemical assay	Mediating relaxation (relative to ATP)[2]			Mediating contraction (relative to ATP)[2]		
	Turkey erythrocyte[1]	Guinea-pig taenia	Rabbit aorta	Rabbit mesenteric artery	Rabbit saphenous artery[3]	Guinea-pig vas deferens	Guinea-pig bladder
Ribose modifications							
19. 2′-Deoxy-ATP	19,200 ±6,200	= 5.6	na	na	na	na[5]	na
20. 3′-Deoxy-ATP	75,500 ±14,800	− −	na	na	na	=	−
21. 2′,3′-Dideoxy-ATP	70,800	= (5.0)				=	+
22. 3′-Amino-3′-deoxy-ATP[5]	193	= (5.4)	+ + (6.4, ≈max)	na	na	+	=
23. 3′-Acetylamino-3′-deoxy-ATP[5]	≫100,000	na	na	+ (<max)	na	=	+
24. 3′-(4-Hydroxyphenylpropionylamino)-3′-deoxy-ATP[5]	≫100,000	na	+ + (<max)	na	na	=	+
25. 3′-Benzylamino-3′-deoxy-ATP	≫100,000	− −	na	na	na	+ +	+ +
26. Isopropylidene-ATP	201,000 ±63,000	− −	na	− −	= [7.7%]	na	− −
27. Isopropylidene-AMP[5]	≫100,000	na	+ + (<max)	na	na	na	na

[1] EC_{50} values (nM) for stimulation of production of inositol phosphates, expressed as the mean ± S.D. for at least 3–5 determinations, or % stimulation at concentration indicated; other symbols as in footnote 2. [2] + +, significantly more potent than ATP; +, more potent than or equal to ATP; =, equal to ATP; −, less potent than or equal to ATP; − −, significantly less potent than ATP; na, not active at the highest concentration tested (usually around 10^{-5} M). Numerical value, if given, is pD_2 in minus log molar units, and for some compounds in rat aorta and mesenteric artery, maximum relaxation relative to 2meSATP (<, ≈, or >) is indicated in parentheses. [3] For the saphenous artery, the percentages in brackets are responses at 10 μM relative to the contraction produced by 1 μM αβmeATP. The highest concentrations tested were 3–10 μM. [4] 6.2 ± 0.08 ($n = 38$). [5] Data for smooth muscle from literature reports (Burnstock *et al.*, 1983, 1984; Cooper *et al.*, 1989; Cusack and Hourani, 1990; Jacobson, 1990). [6] Contraction, not relaxation. [7] Relaxation, but pD_2 not calculable. [8] Compound 11b was approximately 100 times more potent than ATP in the bladder, but it produced tonic contractions rather than the phasic contractions of ATP. In the presence of indomethacin (1 μM), it was much less potent than ATP under the same conditions. Modified from Burnstock *et al.* (1994).

P2Y-purinoceptor heterogeneity, with purinoceptors in the taenia coli and those in the vasculature being recognised differently by ATP derivatives carrying selective structural modifications.

Similarly, heterogeneity of P2X purinoceptors is also suggested. Ribose modifications leading to 3-benzylamino-3′-deoxyATP resulted in a very potent agonist at the P2X purinoceptors in guinea-pig vas deferens and bladder, with no activity in rabbit saphenous artery and at P2Y purinoceptors; this would suggest that the vascular P2X purinoceptor differs from vas deferens and bladder P2X purinoceptors, which appeared to be very similar to each other. This conclusion is also supported by the fact that a number of synthesised derivatives displayed no activity at the saphenous artery P2X purinoceptor, but could not distinguish between the vas deferens and urinary bladder P2X purinoceptors (Table 3). Interestingly, the only compound distinguishing between the vas deferens and the bladder P2X purinoceptor was the pyrimidine compound 5-fluoro-UTP. Whether this is indicative of a true pyrimidine receptor or whether the P2X purinoceptor in the bladder also recognises pyrimidine compounds has yet to be evaluated.

It is apparent from the structure–activity data given in Table 3 that the P2 purinoceptors in the seven tested tissues are all different from one another in their pharmacological profile. This would suggest, therefore, that within the P2X- and P2Y-purinoceptor families there are further subtypes that can be distinguished by the selective actions of ATP derivatives.

4. NEW PROPOSALS FOR P2-PURINOCEPTOR CLASSIFICATION

4.1. P2-Purinoceptor Coupling to Different Transduction Mechanisms: A Criterion for Distinguishing the P2X-(Ligand-Gated) and P2Y-(G-Protein-Coupled) Purinoceptor Families

There is now good evidence to suggest that the P2X- and P2Y-purinoceptor subtypes differ not only in terms of pharmacological profile and tissue distribution, but also in their transduction mechanisms and effector systems. We propose, therefore, the existence of P2X- and P2Y-purinoceptor families, each with their own subdivisions.

Evidence to date suggests that P2X purinoceptors are intrinsic ionic channels permeable to Na^+, K^+ and Ca^{2+}, whereas P2Y purinoceptors are G-protein-coupled receptors. A common feature of the P2Y-purinoceptor family is the activation, following receptor binding by selective agonists, of a membrane shuttle protein belonging to the GTP-binding protein family (G-proteins), which, in turn, activates selective enzyme effector systems that regulate intracellular concentrations of second messengers (for a review, see Gilman, 1987). In the case of P2Y purinoceptors, effector systems are represented by phospholipase C (which modulates IP3 and DAG formation), phospholipase A2 (with consequent generation of arachidonic acid metabolites), or adenylate cyclase (which modulates cAMP levels) (Fredholm *et al.*, 1994). There is now sufficient data to suggest a further subdivision of P2X and P2Y purinoceptors into subclasses (see Section 3), and based on this, we can hypothesise the existence of P2X-(ligand-gated) and P2Y-(G-protein-coupled) purinoceptor *families*.

4.2. Do the P_{2t}, P_{2D} and P_{2u} Receptors Belong to the P2Y-Purinoceptor Family?

As reported in Table 1, the so-called P_{2t}, P_{2D} and the P_{2u}/P_{2n}/'pyrimidine' receptors share with 'classic' P2Y purinoceptors the characteristic of utilizing G-proteins for their transduction mechanisms.

Based on this and other evidence, we would like to propose the inclusion of these purinoceptor subtypes within the P2Y-purinoceptor family.

All present and yet-to-be identified receptors belonging to this P2Y-purinoceptor family would involve G-protein activation, and would be named $P2Y_1$, $P2Y_2$, $P2Y_3$ ... $P2Y_n$, with the clear advantages of eliminating the confusion generated by the 'D', 't' and 'u' subheadings and of easily accommodating any 'new' P2Y-purinoceptor subtype by simply assigning it a progressive number.

Apart from the G-protein involvement, there are a number of additional reasons to support this new way of defining the different ATP receptor subtypes.

In the case of the P_{2u}/'nucleotide' receptor, such a classification would be supported by the fact that this receptor subtype recognises both UTP and ATP and has in common with the recently

Table 4. *Proposed Subclassification of P2 Purinoceptors*

Name	P2X-purinoceptor family	P2Y-purinoceptor family[1]	P2Z
Type	Ligand-gated channel	G-protein-coupled	Non selective pore
General agonist profile	$\alpha\beta$meATP > $\beta\gamma$meATP > ATP ≈ 2meSATP ≈ ADP	2meSATP > ATP = ADP > $\alpha\beta$meATP ≥ $\beta\gamma$meATP	ATP^{4-}
Antagonists	$\alpha\beta$meATP desensitisation Suramin Selectively blocked by PPADS ANAPP3	Suramin Reactive blue 2	Oxidised ATP

[1]It is proposed that the P2Y family also includes the former G-protein-linked P_{2u}/P_{2n}/pyrimidine receptor (where UTP ≥ ATP), the P_{2t} purinoceptor (where ADP is the unique agonist) and the P_{2D}-purinoceptor (where ApxA represents the specific agonists: see text for details).

cloned $P2Y_1$-purinoceptor sequences encoding for the same group of G-protein-coupled receptors (Section 2).

For the P_{2t} receptor, inclusion into the P2Y-purinoceptor family as $P2Y_3$ is proposed following the isolation of a related receptor (clone 103, Barnard *et al.*, 1994) during the cloning work with chick brain, which led to the isolation of the $P2Y_1$ purinoceptor (clone 803, Webb *et al.*, 1993).

For the P_{2D} receptor subtype, inclusion into the P2Y-purinoceptor family would be justified by the fact that, although the major agonists of this subclass are dinucleotides rather than mononucleotides, this receptor subtype displays a 'P2Y-like' pharmacological profile in chromaffin cells and *Torpedo* synaptic terminals (Pintor and Miras-Portugal, 1993). As summarised in Table 4, acquisition of this new nomenclature would lead to a simpler purinoceptor classification comprising the major divisions P2X/P2Y/P2Z.

To support our hypothesis, we have analyzed carefully a number of papers published between 1987 and 1994 on the pharmacological actions of ATP and its analogues. Particular attention has been paid to comparative studies where, besides ATP itself, a variety of different ATP analogues have been tested, in an attempt to assess whether specific pharmacological profiles could be defined in different tissues and systems.

4.3. Proposals for the Subclassification of the P2Y-Purinoceptor Family

By carefully analysing the studies published so far on P_{2Y}-like responses, we were able to define at least seven different agonist potency profiles, which, indeed, would suggest the existence of seven P2Y-purinoceptor subclasses (Table 5).

Two major considerations need to be taken into account before describing in detail the proposed P2Y-purinoceptor subtypes. First, examples listed in Table 5 only refer to cases in the literature where all the key agonists selected by us to establish the rank order of potency for the putative $P2Y_{1-7}$ purinoceptor subtypes have been tested. Of course, there are many more examples of P2Y-like responses in a variety of other tissues or systems not listed in Table 5, where, however, only some of the key compounds have been tested, therefore making it impossible to 'assign' the tissue to a specific receptor subtype. Second, the reader might notice that some tissues are listed twice under two different P2Y-purinoceptor subtypes. Whether this is due to the co-presence of multiple purinoceptor populations in the same tissues or to different tissue preparations and/or animal species is difficult to say at the moment. The use, where available, of molecular probes for the P2Y-purinoceptor subtypes and of the new selective P2Y agonists (Section 3) will be of help in clarifying this aspect.

The $P2Y_1$ receptor recently cloned by Webb *et al.* (1993) shows the following agonist potency profile: 2meSATP ≥ ATP ≫ ADP ⋙ $\alpha\beta$meATP. We were able to find several examples of systems displaying this agonist potency profile (see list in Table 5). In some cases, pharmacological data correlated well with expression of the chick $P2Y_1$ transcript, as determined in Webb and coworkers' study by Northern hybridisation. This would suggest strongly that the clone identified by Webb *et al.* is the P2 purinoceptor independently studied by other researchers showing this typical agonist

Table 5. *Proposed Subclassification of the P2Y G-Protein-Linked Purinoceptor Family*

Proposed subtype	$P2Y_1$	$P2Y_2^*$	$P2Y_3$†	$P2Y_4$	$P2Y_5$	$P2Y_6$	$P2Y_7$‡
Agonist potency	2meSATP≥ ATP≫ADP> ≫αβmeATP	ATP≥UTP= ATPγS; UTP> ATP only for footnotes 1–3	2meSADP> ADP	2meSATP≫ ATP=ADP= αβmeATP > ≫ βγmeATP	2meSATP≥ ATP=ADP > ≫αβmeATP βγmeATP inactive	2meSATP> ATP>ADP?	Diadenosine polyphosphates
Selective agonists	2meSATP	UTPγS		2′-deoxy-ATP and also N^6-methyl-ATP selective for *taenia coli*	8-(6-aminohexylamino)-ATP and ATP-N-oxide selective for *endothelial cells*	No selective agonists available yet; however, the compounds selective for P2Y4 and P2Y5 are inactive on *vascular smooth muscle*	
Name of clone	Clone 803 (Webb *et al.*, 1993)	Clone pP2R (Lustig *et al.*, 1993) Clone HP2U (Parr *et al.*, 1994) Clone from rat heart tissue, (Godecke and Schrader, 1994)	Brain-derived clone 103[4]				
Number of amino acids	362	373 for clone pP2R 375 for clone HP2U					

Proposed subtype	$P2Y_1$	$P2Y_2^*$	$P2Y_3$†	$P2Y_4$	$P2Y_5$	$P2Y_6$	$P2Y_7$‡
Tissue	Chick brain[5] Rat brain cortex[6] Developing chick skeletal muscle?[5,7] Guinea-pig cochlear hair cells?[8] Rabbit gastric glands?[9]	Rat renal mesangial cells[10,11] Rat mesenteric arterial bed[12] Rat aortic smooth muscle cells[13] Rat liver[11,14] Rat hepatocytes[11,15] Rat osteoblastic cells[16] Rabbit[17] and human?[18] neutrophils Guinea-pig brain[19] Sheep pituitary cells[20] Rat myocardial endothelium[1,11] and bovine endothelial cells[21] Skeletal muscle[11] Human fibroblasts[22] Human amnion cells[2,11] Human airway epithelial cells[11,23] HL60 cells[24–26] PC12 cells[27] Mouse neuroblastoma cells[28]	Platelets[29] Megakaryocytes[30] Brain capillary endothelial cells?[31]	Guinea-pig taenia coli[3] Turkey erythrocytes[32–35]	Rabbit aorta[35] Pig aorta?[36] Rat cortical astrocytes[37,38] Organ of Corti cells[39] Dorsal spinal cord astrocytes[40]	Rabbit coronary[41], hepatic[42] and mesenteric[35] arteries Human subcutaneous and omental resistance arteries?[43] Human endothelial cells?[44]	Rat brain synaptosomes[45] Rat cortical neurons[46] Chromaffin cells[47–49] Porcine[50] and bovine[51] aortic endotheli al cells Human platelets[52–54] Mammalian hepatocytes[55] Guinea-pig vas deferens, urinary bladder[56] and isolated arteries[57]

*Formerly P_{2u} or P_{2n} or 'nucleotide' receptor. †Formerly P_{2t}. ‡Formerly P2D. [1]As suggested by hybridisation studies with rat heart mRNA (Godecke and Schrader, 1994). [2]Vander Kooy *et al.* (1989). [3]Satchell and Maguire (1975), Maguire and Satchell (1979), Burnstock *et al.* (1983), Cusack *et al.* (1987). [4]Two quite different full-length cDNAs were isolated by Webb *et al.* (1993); one encoding for the $P2Y_1$ purinoceptor, the other encoding for a receptor responsive to ADP (Barnard *et al.*, 1994). Preliminary data suggest that this second recombinant brain-derived receptor is similar, but not identical, to the P_{2t} receptor of mammalian platelets. This is also consistent with Frelin *et al.* (1993). [5]Also supported by hybridisation with $P2Y_1$ mRNA (Webb *et al.*, 1993). [6]von Kugelgen *et al.* (1994); first description of presynaptic P2 purinoceptors in the CNS. [7]Thomas *et al.* (1991). [8]Nakagawa *et al.* (1990). [9]Gil-Rodrigo *et al.* (1990). [10]Reactive blue 2-dependent effect; clear evidence for G-protein involvement; no evidence for separate ATP and UTP receptors; Pfeilschifter (1990). [11]As shown by hybridisation with HP2U mRNA (Parr *et al.*, 1994). [12]Ralevic and Burnstock (1991). [13]Pertussis-toxin sensitive effect (Tawada *et al.*, 1987). [14]Haussinger *et al.* (1987). [15]Keppens *et al.* (1992). [16]Clear evidence for a common UTP/ATP receptor-mediating release of Ca^{2+} from internal stores; these cells also have a receptor to ADP (Reimer and Dixon, 1992). [17]Elferink *et al.*, (1992). [18]Pertussis toxin-sensitive effect (Walker *et al.*, 1991). [19]As shown in *Xenopus* oocytes injected with guinea-pig brain mRNA (Honore' *et al.*, 1991). [20]Phospholipase C-mediated effects (Davidson *et al.*, 1990). [21]Phospholipase C-mediated effects; common UTP/ATP receptor suggested (Purkiss *et al.*, 1993). [22]Fine *et al.* (1989). [23]Evidence for a common ATP/UTP receptor coupled to phospholipase C (Mason *et al.*, 1991; Brown *et al.*, 1991); nature of this receptor has been confirmed recently by functional expression of the cloned human receptor in 1321N1 cells (Lazarowski *et al.*, 1994). [24]Xing *et al.* (1992). [25]Cockcroft and Stutchfield (1989). [26]Also shown in *Xenopus* oocytes injected with mRNA from HL60 cells (Murphy and Tiffany, 1990). [27]Evidence for an ATP/UTP phospholipase-linked receptor (Murrin and Boarder, 1992); however, it is not clear whether multiple receptors for ATP and UTP are expressed in these cells (Reed *et al.*, 1994). [28]As shown by hybridisation with pP2R mRNA (Lustig *et al.*, 1993). [29]Cusack *et al.* (1979). [30]Burnstock (1991). [31]ADP-specific receptor inducing Ca^{2+} mobilisation from a thapsigargin-sensitive intracellular pool (Frelin *et al.*, 1993). [32]Berrie *et al.* (1989). [33]Boyer *et al.* (1990). [34]2-(6-cyanohexylthio)-ATP shows nanomolar potency at the turkey erythrocyte P2Y receptor (Fischer *et al.*, 1993). [35]Burnstock *et al.* (1994). [36]Martin *et al.* (1985). [37]Phospholipase C-linked receptor (Kastritsis *et al.*, 1992). [38]Activation of this receptor leads to intracellular Ca^{2+}-dependent arachidonic acid release (Bruner and Murphy, 1990). [39]Dulon *et al.* (1993). [40]Activation of this receptor leads to mobilisation of Ca^{2+} from a thapsigargin-sensitive intracellular store (Salter and Hicks, 1994). [41]Corr and Burnstock (1991). [42]Brizzolara and Burnstock (1991). [43]$\alpha\beta$meATP practically inactive (Martin *et al.*, 1991). [44]Carter *et al.* (1988) reported that activation of this receptor on human cultured umbilical vein endothelial cells induces intracellular Ca^{2+}-dependent prostacyclin secretion. Evidence was produced in favour of the different nature of this receptor from the taenia coli P2Y purinoceptor. [45]Pintor *et al.* (1993). [46]Stone and Perkins (1981). [47]Pintor *et al.* (1991a). [48]Pintor *et al.* (1991b). [49]Castro *et al.* (1992). [50]Goldman *et al.* (1986). [51]Ogilvie *et al.* (1989). [52]Flodgaard and Klenow (1992). [53]Luthje and Ogilvie (1983). [54]Zamecnik *et al.* (1992). [55]Rapaport and Zamecnik (1976). [56]Hoyle *et al.* (1989); Hoyle (1990). [57]Busse *et al.* (1988).

potency profile. In other cases, there was a clear, although not yet conclusive, matching between the pharmacological data and the results of the hybridization studies with $P2Y_1$ mRNA. For example, Thomas *et al.* (1991) reported the presence of a P2 purinoceptor (whose pharmacological profile was not unequivocally assessed) in chick skeletal muscle, a tissue that was shown to abundantly express the $P2Y_1$ transcript. However, it is not clear if the receptor described by Thomas *et al.* is a G-protein-linked receptor or a ligand-gated cation channel.

In analysing the abundant literature on the UTP-sensitive receptor, we could identify two receptor behaviour patterns: in most cases, UTP was equipotent with ATP, whereas in a few other examples, UTP was more potent than ATP. At present, there is not sufficient data to support the existence of two different UTP-sensitive purinoceptors (the first one preferentially responding to ATP and the second one preferentially responding to UTP), and we, therefore, put together the two agonist behaviour patterns and named this purinoceptor $P2Y_2$ (Table 5). UTPγS seems to behave as a selective agonist at this receptor subtype (Table 5).

Based on our proposal, clone pP2R (Lustig *et al.*, 1993), therefore, would be the $P2Y_2$-purinoceptor subtype. The detection of the pP2R transcript in kidney, liver, lung, heart and brain (Lustig *et al.*, 1993) matches well with the reported presence of the $P2Y_2$ purinoceptor in rat renal mesangial cells (Pfeilschifter, 1990), rat hepatocytes (Keppens *et al.*, 1992), human airway epithelial cells (Mason *et al.*, 1991; Brown *et al.*, 1991; Lazarowski *et al.*, 1994) and guinea-pig brain (Honoré *et al.*, 1991; Table 5). As underlined in Table 5, a good correlation from previous literature reports and expression of receptor mRNA was also found for the recently cloned HP2U purinoceptor (Parr *et al.*, 1994). In the examples listed in Table 5, the effects of ATP and UTP do not seem to be additive when both agents are utilised at maximal doses suggesting the presence of a *common* ATP/UTP receptor (references 10, 15, 16 and 23 of Table 5), in agreement with the conclusion recently drawn by Keppens (1993) in reviewing the effects of ATP and UTP in isolated hepatocytes. Moreover, where tested, UTP responses appear to be antagonised by reactive blue 2 or suramin, which are known to act as antagonists at 'classic' P_{2Y} purinoceptors (Hoyle *et al.*, 1990). Both these characteristics further strengthen the inclusion of this receptor into the P_{2Y}-purinoceptor family.

Nonetheless, there may be a need to recognise new classes of receptor associated with the P2 families that are not yet included in our proposed classification. For example, recent data from the C6-2B rat glioma cell line identified a phospholipase C-linked receptor, which was claimed to be selectively activated by UTP and related uridine nucleotides, but showed insensitivity to ATP, thus differentiating it from the pP2R and HP2R clones (Lazarowski and Harden, 1994). This receptor may represent a new class of 'P_2-like' receptor. For the long term, the resolution of whether there are distinct $P2Y_2$ ('P2U') and 'UTP' (pyrimidine) receptors will be dependent on the generation of new data, both molecular and pharmacological, that will unambiguously delineate the nature of the receptor subtypes responding to UTP (Williams, 1994).

The currently named P_{2t} receptor ($P2Y_3$ in our proposed subclassification) seems to be expressed exclusively by platelets and megakaryocytes. However, we were able to find a very recent report of a similar ADP receptor in brain capillary endothelial cells (Frelin *et al.*, 1993), which would suggest a wider biological role for this receptor subtype. The possible presence in brain of a P2-purinoceptor subtype displaying a strong preference to ADP is also suggested by the second recombinant brain-derived receptor recently cloned by the Barnard/Burnstock London group and obtained during the polymerase chain reaction amplification of sequences encoding G-protein-coupled receptors, which led to the cloning of the $P2Y_1$-purinoceptor subtype (Webb *et al.*, 1993). This second clone, designated $P2Y_3$, is largely similar (although not identical) to the P_{2t} purinoceptor of mammalian platelets, and Northern hybridisation studies have revealed the presence of this mRNA in brain and several peripheral tissues (Barnard *et al.*, 1994). These results confirm that the currently named P_{2t} receptor also belongs to the G-protein-coupled receptor superfamily and underlines its close relationship to the P2Y-purinoceptor family.

In our analysis of the literature in this area, we also found examples in favour of three additional agonist potency profiles: 2meSATP $\gg$ ATP = ADP = $\alpha\beta$meATP $\ggg$ $\beta\gamma$meATP ($P2Y_4$), 2meSATP $\geqslant$ ATP = ADP $\ggg$ $\alpha\beta$meATP ($\beta\gamma$meATP inactive) ($P2Y_5$), and 2meSATP > ATP > ADP ($P2Y_6$). These three additional receptors can now be discriminated from each other with the aid of newly synthesised compounds.

The prototype for the $P2Y_4$ subtype is represented by the guinea-pig taenia coli, and this receptor

seems to be selectively activated by the new compounds 2′-deoxy-ATP and N^6-methyl-ATP (Table 5 and Section 3). A characteristic of this receptor is the much higher sensitivity to 2meSATP with respect to ATP. For example, 2meSATP is 250-fold more potent than either ATP or ADP in stimulating phosphatidylinositol breakdown in turkey erythrocytes (Berrie *et al.*, 1989).

The prototype for the $P2Y_5$ subtype is represented by the endothelial receptor mediating blood vessel relaxation via generation of nitric oxide. The new compounds 8-(6-aminohexylamino)-ATP and ATP-*N*-oxide, which were shown to be selective for aortic endothelial cells (Burnstock *et al.*, 1994), could behave as selective $P2Y_5$ purinoceptor agonists and will be useful tools to be tested in the biological systems displaying a similar agonist potency profile (Refs 37–40 of Table 5), which, besides endothelial cells, also include astrocytes and Organ of Corti cells.

The $P2Y_6$ purinoceptor is the vascular smooth muscle receptor responsible for direct ATP vasodilation (Refs 35, 41 and 42 of Table 5). No selective agonists are available yet for this purinoceptor subtype. However, derivatives selective for the $P2Y_4$ and $P2Y_5$ subtypes are inactive at this receptor subtype. Again, future studies aimed at testing the activity of the new compounds in a variety of tissues will confirm the identity and nature of this hypothetical purinoceptor subtype.

Interestingly, one of the newly synthesised analogs, 2-(6-cyanohexylthio)-ATP, was proven to be extremely potent at both the guinea-pig taenia coli and the rabbit aorta and the rabbit mesenteric artery ($P2Y_4$–$P2Y_6$) purinoceptor subtypes (Fischer *et al.*, 1993; Burnstock *et al.*, 1994). This compound belongs to the family of long-chain 2-alkylthio-ATP derivatives, which were previously shown to resist degradation by nucleotidases (Zimmet *et al.*, 1993) and may serve as the basis for the design of useful molecular probes for ATP receptors.

Finally, an additional purinoceptor subtype included in this receptor family is the $P2Y_7$ purinoceptor (formerly P_{2D}), the presence of which has been demonstrated in a variety of systems (Refs 45–57 of Table 5). The reasons for assigning this receptor to the P2Y-purinoceptor family have been explained previously (Section 4.2) and, again, a definite confirmation, or not, that this represents a P2Y-purinoceptor subtype distinct from the others will come from cloning data.

4.4. Proposals for the Subclassification of the P2X-Purinoceptor Family

The use of new ATP derivatives with modified purine, ribose or triphosphate moieties suggests heterogeneity within the P2X-ion-gated purinoceptors (Burnstock *et al.*, 1994). This heterogeneity is also consistent with the results of our analysis of a series of literature papers reporting P2X-like responses. Again, the references reported here refer to studies where a number of key agonists have been tested and, therefore, this review cannot be comprehensive for all the papers published on P2X-purinoceptor-mediated effects.

Although evidence for subclassification in this case was less obvious than that of the P2Y purinoceptor, there are indications for at least four different agonist potency profiles, leading to the identification of four P2X-purinoceptor subclasses (Table 6).

Guinea-pig vas deferens seems to be the prototype of the first P2X purinoceptor subtype. In general, at this receptor, aβmeATP is equipotent with ATP in inducing contraction (Burnstock *et al.*, 1994; Wiklund and Gustafsson, 1988). This receptor recently has been reported to have been cloned by the Glaxo group (A. Surprenant, personal communication*), and, therefore, it has been named it $P2X_1$. It will be, of course, extremely interesting to test the new ATP derivatives shown to be selective for vas deferens (Section 3) on the cloned transfected receptor. Useful information is likely to come in the near future from an analysis of the distribution of its mRNA in the other tissues showing P2X-like responses.

The pharmacology of the urinary bladder P2X purinoceptor appears to be different from that of the vas deferens receptor. In both guinea-pig (Burnstock *et al.*, 1994; Cusack, 1993) and rat urinary bladder (Bo *et al.*, 1994) both $\alpha\beta$meATP and $\beta\gamma$meATP are significantly more potent than ATP and 2meSATP. The different nature of this receptor is also confirmed by the fact that it can be discriminated from the vas deferens receptor with the new derivative 5-fluoro-UTP (Section 3; Table 6). Both this compound and the other new derivatives reported in Tables 3 and 6 will be invaluable experimental tools to test the presence of this receptor subtype (named $P2X_2$) in a number of systems.

*See Valera *et al.* (1994) in "Note added in proof".

Table 6. *Proposed Subclassification of the P2X-Ion Gated Purinoceptor Family*

Proposed subtype	$P2X_1$	$P2X_2$	$P2X_3$	$P2X_4$
Selective agonists	2-(4-nitrophenylethyl-thio)ATP > 3′-amino-3′ -deoxy-ATP = 2-hexyl-thioATP = 2-cyclo-hexyl-thioATP	5-fluoro-UTP > 2-hexyl-thioATP = 3′-acetylamino -3′-deoxy-ATP > 3′(4-hydroxy-phenylproprionyl-amino)-3′-deoxy-ATP	No selective agonists available; however, the compounds selective on the other subtypes are inactive	No selective agonists available
Tissues	Guinea-pig vas deferens[1-3]	Guinea-pig[1,4] and rat[5] urinary bladder Rabbit bladder detrusor[6] Rat colon longitudinal muscle?[7]	Vascular smooth muscle[1]	Peripheral[8-12] and central[13-16] neurons Ventricular myocytes[17] Microglial cells[18]

[1]Burnstock *et al.* (1994). [2]Wiklund and Gustafsson (1988). [3]A. Surprenant, personal communication. [4]Cusack (1993). [5]Bo *et al.* (1994). [6]Kishii *et al.* (1992). [7]Bailey and Hourani (1992). [8]Bean (1990). [9]Allen and Burnstock (1990). [10]Krishtal *et al.* (1983). [11]Fieber and Adams (1991). [12]Surprenant (1994). [13]Ueno *et al.* (1992). [14]Edwards *et al.* (1992). [15]Tschopl *et al.* (1992). [16]Shen and North (1993). [17]Bjornsson *et al.* (1989). [18]Langosch *et al.* (1994).

A third P2X-purinoceptor subclass ($P2X_3$) might be represented by the receptor expressed in vascular smooth muscle. At the saphenous artery P2X purinoceptor, 2meSATP and the new derivatives selective for the $P2X_1$ and $P2X_2$ are inactive (Burnstock *et al.*, 1994; Table 6). It would be interesting to assess the similarities of this receptor with that described by O'Connor *et al.* (1990) in rabbit ear artery. No selective agonists for this receptor subtype are available yet.

An additional P2X-purinoceptor subtype ($P2X_4$) might be represented by the neuronal ATP-activated cation channel described in peripheral and central neurons (references 8–16 in Table 6). The general order of potency for ATP analogues in activating this receptor is 2meSATP > ATP > $\alpha\beta$meATP (Illes and Norenberg, 1993). A similar behaviour is displayed by ATP derivatives also in ventricular myocytes (reference 17 in Table 6) and rat microglial cells (reference 18 in Table 6). The different nature of this receptor from the other hypothesised P2X-purinoceptor subtypes is confirmed by preliminary results, suggesting that the transfected $P2X_1$ purinoceptor clone behaves differently in terms of pharmacological response from the neuronal ATP-sensitive channel (A. Surprenant, personal communication).

Finally, there are a number of reports in favour of the existance of excitatory purinoceptors responding to both ATP and UTP (von Kugelgen *et al.*, 1987, 1990; von Kugelgen and Starke, 1990; Saiag *et al.*, 1990, 1992; Theobald, 1992; Hourani *et al.*, 1993; Pavenstadt *et al.*, 1991; Rubino and Burnstock, 1994; Ralevic and Burnstock, 1991). This receptor differs from the proposed $P2Y_2$ purinoceptor ($=P_{2u}$ purinoceptor), since apparently it is not coupled to G-proteins, but mediates rapid and transient responses (usually excitatory). In some instances and tissues, evidence was produced in favour of a *common* ATP/UTP receptor (von Kugelgen *et al.*, 1990; Hourani *et al.*, 1993; Ralevic and Burnstock, 1991); in other cases, distinct UTP receptors were hypothesised (Saiag *et al.*, 1990, 1992; von Kugelgen *et al.*, 1987). Whether this receptor really represents a distinct entity with respect to the other P2X-purinoceptor subtypes cannot be specified at this time, also because UTP was not tested in all the other studies reported for receptors $P2X_1$–$P2X_4$. Here again help will come from molecular cloning studies.

We are aware of the fact that differences of agonist order potency within different tissues may partially derive from different ecto-ATPase activities (Cusack, 1993). In this respect, useful and crucial information in terms of purinoceptor classification could come either from the use of dephosphorylation-resistant agonists (e.g. L-$\beta\gamma$-methylene-ATP; Cusack, 1993) or by utilising inhibitors of nucleotide degradation (Leff *et al.*, 1994; Ziganshin *et al.*, 1994a).

5. P2-PURINOCEPTOR LIGANDS AS POTENTIAL THERAPEUTIC ENTITIES

While there have been several excellent reviews on the potential development of P1-purinoceptor targeted drugs (Daly, 1982; Daval *et al.*, 1991; Jacobson *et al.*, 1992; Stone, 1992; Williams, 1993), there has been very little discussion on the possible clinical and therapeutic applications of P2-purinoceptor agonists and antagonists (Burnstock, 1993).

Due to the recent explosion of interest in P2 purinoceptors, which has enormously advanced our knowledge on the functional roles of ATP in many different organs and systems, we now have enough experimental data to suggest that P2 purinoceptors might also represent a novel target for drug development in a variety of pathological conditions.

Moreover, the large heterogeneity of P2-purinoceptor subtypes in the different tissues underlined in this review opens the possibility of developing highly selective organ- or tissue-specific P2-purinoceptor-targeted drugs. Several exciting possibilities are being explored.

5.1. Diabetes

A number of studies has demonstrated the presence of P2Y purinoceptors on insulin-secreting pancreatic β-cells (Bertrand *et al.*, 1987; Arkhammar *et al.*, 1990; Li *et al.*, 1991; Hillaire-Buys *et al.*, 1992, 1993). The activation of this receptor leads to potent phospholipase C-mediated intracellular Ca^{2+} mobilisation, sustained insulin secretion and notable improvement of glucose tolerance. Interestingly, not only was the ectonucleotidase-resistant P2Y-purinoceptor agonist ADPβS demonstrated to be effective after oral administration (Hillaire-Buys *et al.*, 1993), but this agent also retained its insulin-stimulatory effects in streptozotocin-diabetic rats (Hillaire-Buys *et al.*, 1992).

These findings suggest that the P2Y purinoceptor represents a novel target for the development of oral antidiabetic drugs.

5.2. Cancer

Based on the potent growth inhibition of a variety of human and murine tumour cells by extracellular ATP, the administration of AMP or ATP to tumour-bearing murine hosts was shown to be associated with dramatic cytostatic and cytotoxic effects (Rapaport, 1993). Such anticancer activity is likely related to the activation of P2Z purinoceptors mediating cell killing through apoptosis (Pizzo *et al.*, 1993; Murgia *et al.*, 1992). The utilisation of ATP infusions against metastatic refractory cancers has already entered Phase I of human clinical trials. This clinical application is particularly intriguing since studies in cachectic tumor models have shown that the ATP-mediated tumor-killing activity is also associated with a variety of host-mediated anticancer activities, including significant inhibition of host weight loss (Rapaport, 1993).

5.3. Cystic Fibrosis

P2 purinoceptors have been shown to regulate ion transport in epithelial cells from a variety of different sources, including intestinal, lung and kidney epithelium, where ATP stimulates Cl^- transport and alters Ca^{2+} distribution (Burnstock, 1991). Cystic fibrosis (CF) is a lethal genetic disease characterised by defective regulation of chloride conductance in airway epithelia, which leads to the formation of underhydrated mucus obstructing the airways of patients with this disease (Noone and Knowles, 1993). Both ATP and UTP were found to be effective *in vivo* Cl^- secretagogues in the nasal mucosa of human subjects, an effect that seemed secondary to the activation of phospholipase C and Ca^{2+}-dependent phosphorylation of Cl^- channels (Stutts *et al.*, 1992). Most interestingly, regulation of Cl^- conductance by ATP and UTP was preserved in CF nasal epithelia (Clarke and Boucher, 1992). On the basis of these results, it is intriguing to speculate that agonists at this P2-purinoceptor subtype (a $P2Y_2$ receptor?) might be of enormous clinical interest in normalising electrolyte and water secretion across CF airway epithelia.

5.4. Pulmonary Hypertension

In the lung, the most interesting target for drug development is represented by the vascular pulmonary P2 purinoceptor mediating ATP-induced vasoconstriction (McCormack *et al.*, 1993). This receptor seems different from the typical P2X purinoceptor responsible for excitatory (vasoconstrictor) responses, being equally activated by ATP and UTP (Rubino and Burnstock, 1994). The demonstration of a new purinoceptor involved in the control of pulmonary circulation is particularly intriguing, especially in view of the fact that attempts to lower pulmonary vascular resistance with pharmacological agents have been largely unsuccessful (McCormack *et al.*, 1993). Based on this, antagonists at the ATP/UTP vasoconstrictor lung purinoceptor might have therapeutic potential in hypoxic pulmonary vasoconstriction and in pulmonary hypertension.

5.5. Surfactant and Mucin Secretion

ATP also affects additional lung functional parameters. ATP has been demonstrated to increase mucin release from cultured airway goblet cells through the production of inositol phosphate (Kim *et al.*, 1993), suggesting a role for P2Y purinoceptors in maintaining lung visco-elastic properties and, consequently, in the defence against airborne particles. Phospholipase C-linked P2 purinoceptors also have been demonstrated to be expressed by Type II pneumocytes, the lung cell type responsible for surfactant secretion (Griese *et al.*, 1993). Activation of this receptor leads to increases of surfactant secretion into the bronchial lumen. Based on the hypothesis that, in some forms of pneumonia (Lachmann and Gommers, 1993), the decreased respiratory performance can be attributed to deficiency of surfactant secretion, it would be possible to propose the use of ATP analogs to optimise surfactant secretion in these pathologies.

5.6. Renal Failure

Acute renal failure is commonly provoked by renal ischaemia. Current therapeutic strategies to improve renal blood flow are limited to resuscitation of the systemic circulation and infusion of low doses of dopamine. Failure of this line of management frequently leaves the patient requiring haemodialysis. P2X purinoceptors on the vascular smooth muscle of pre-glomerular (afferent) arterioles can represent an obvious target for antagonists that could enhance renal blood flow and help restore normal renal function. Likewise, analogues of ATP that stimulate P2Y purinoceptors could also increase renal blood flow (Churchill and Ellis, 1993a), although this may be opposed by P2Y purinoceptors mediating renin release (Churchill and Ellis, 1993b).

Renal infusion of ATP–$MgCl_2$ has been demonstrated to enhance post-ischaemic recovery of both glomerular and tubular functions (Siegel *et al.*, 1983; Hirasawa *et al.*, 1985), underlining the possible use of ATP analogues in preventing ischemia-induced renal damage, although the mechanism of action is not fully understood. Beneficial effects of ATP–$MgCl_2$ have been demonstrated also against drug-induced nephrotoxicity (Sumpio *et al.*, 1985) and for kidney preservation before transplantation (Belzer *et al.*, 1983).

Interestingly, exogenous ATP stimulates the proliferation of mesangial cells in glomerulonephritis (Schulze-Lohoff *et al.*, 1992). Specific antagonists of P2 purinoceptors, therefore, may have a therapeutic potential in modulating the anti-inflammatory process of this serious disease.

5.7. Bone and Cartilage Diseases

ATP stimulates cartilage resorption through the activation of a P2 purinoceptor responsive to both ATP and UTP on chondrocytes (Caswell *et al.*, 1991). Interestingly, cytokines, which have been strongly implicated in the loss of cartilage extracellular matrix in rheumatoid arthritis and osteoarthritis, seem to act through enhancement of chondrocyte responsiveness to extracellular ATP (Leong *et al.*, 1993). Similarly, P2 purinoceptors have been demonstrated to be expressed also by osteoblasts (Kumagai *et al.*, 1991; Scholf *et al.*, 1992; Reimer and Dixon, 1992; Yu and Ferrier, 1993) and osteoclasts (Yu and Ferrier, 1993). In osteoblasts, P2 purinoceptor activation by both ATP and UTP has been associated with fast intracellular Ca^{2+} pulses (a $P2Y_2$ receptor?). Since indirect stimulation of osteoclastic resorption is thought to be triggered by an initial elevation of $[Ca^{2+}]i$ in osteoblasts (Reimer and Dixon, 1992), it is intriguing to speculate that the blockade of the osteoblast receptor by selective P2Y-purinoceptor antagonists might be beneficial in pathologies characterised by excessive bone demineralisation such as osteoporosis.

5.8. Urinary Incontinence

ATP has been proposed to be the transmitter responsible for the atropine-resistant contractile response of isolated detrusor muscle elicited by transmural nerve stimulation (Burnstock *et al.*, 1972, 1978). This effect is exerted through the activation of P2X purinoceptors (Burnstock and Kennedy, 1985; Bo and Burnstock, 1990). In interstitial cystitis (a chronic bladder disorder characterised by incontinence), a pathological increase of detrusor muscle sensitivity to ATP analogs was demonstrated recently (Palea *et al.*, 1993), opening interesting possibilities for selective P2X antagonists in the treatment of chronic bladder disorders characterised by dysuria.

5.9. Thrombosis

ATP has been reported to antagonise ADP-induced human platelet aggregation (Table 1; Cusack and Hourani, 1982), which might have interesting implications in the development of anti-thrombotic agents.

5.10. Ventricular Tachycardia

Both P_1- and P_2-purinoceptor antagonists have been recommended for paroxysmal ventricular tachycardia (Belhassen and Pelleg, 1984).

5.11. Gastro-Intestinal Tract

ATP has long been recognised as a nonadrenergic, noncholinergic inhibitory transmitter in the gastro-intestinal tract (Burnstock *et al.*, 1970) and more recently in sympathetic nerves supplying the intestine, where ATP is co-stored and co-released with noradrenaline (for a review, see Hoyle and Burnstock, 1991). P2 purinoceptors (in most cases of the P2Y-subtype, mediating relaxation) so far have been identified in stomach and intestinal smooth muscle, in intestinal ganglia and in endothelial and smooth muscle cells of vessels supplying the gastro-intestinal tract (Hoyle and Burnstock, 1991; Burnstock *et al.*, 1994). In the next few years, these receptors are likely to be explored as possible targets for the development of new pharmacological agents with therapeutic potential in gastro-intestinal dysfunctions.

5.12. Centrally Targeted P2 Purinoceptor Drugs

The recent demonstration of P2 purinoceptors in the CNS (Table 5) also opens interesting opportunities for the development of novel pharmacological agents for the treatment of CNS disorders, such as epilepsy, depression and aging-associated neurodegenerative diseases (Burnstock, 1993; Williams, 1993).

5.13. Human Reproduction

The demonstration of ATP receptors on human spermatozoa (Foresta *et al.*, 1992) and amnion cells (Vander Kooy *et al.*, 1989) may suggest in the near future additional therapeutic implications for P2-purinoceptor agonists and antagonists in human reproduction.

6. FUTURE DEVELOPMENTS

Perhaps the most important area for future research, partly to substantiate our proposed P2-purinoceptor subclassification and also in relation to therapeutic development, is to identify selective antagonists for the different P2-purinoceptor subclasses defined in this review; antagonists that are effective *in vivo* are especially desirable.

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NOTE ADDED IN PROOF

When this paper was already in press, two independent reports appeared in *Nature* on the cloning of two different ionotropic P2X-purinoceptors from rat vas deferens (Valera *et al.*, *Nature* **371**: 516–519) and from PC12 cells (Brake *et al.*, *Nature* **371**: 519–523). The first one is the receptor communicated by Suprenant in the present review. For both receptors, deduced membrane topology was strikingly different from that of other ionotropic transmitter receptors such as the acetylcholine nicotinic receptor. In both cases, deduced amino acid sequence contained two hydrophobic putative transmembrane domains with an intervening hydrophilic cysteine-rich loop of 270–287 amino acids, suggesting that P2X-purinoceptors may belong to an entirely different class of transmembrane signalling proteins. Consistently with our proposal on P2X-purinoceptor heterogeneity, the two receptors revealed different pharmacological profiles when expressed in oocytes and their transcripts showed different tissue distribution as revealed by *in situ* hybridisation analysis.

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